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A REVIEW

Assessment of genetic purity of tomato F1 hybrid seeds with molecular and biochemical markers

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INTRODUCTION

DNA marker is a new approach based on DNA polymorphism among tested genotypes, (John and Williams, 2008), thus, applicable to biological research. It offers many advantages over other categories of markers such as morphological, cytological or biochemical markers. For example, DNA marker can cover the whole genome and, therefore, is much larger in quantity. There is more polymorphism in DNA markers, which are able to reveal the variation and allelism. Many DNA markers are co-dominant and can differentiate between the homozygous and heterozygous genotypes. Furthermore, DNA markers are "neutral", and they have no effect on phenotype, no epistatic effect, and are not influenced by environmental conditions and development stages. Therefore, DNA marker is simple, quick, less environmentally conditioned, and experimentally reproducible well. It has been applied widely in the identification, registration of plant variety, and in monitoring of the seed purity and the authenticity with high accuracy, high reliability and low cost. However, due to rapid developments in marker technology, statistical methodology for identifying quantitative trait loci (QTLs) and the jargon used by molecular biologists, the utility of DNA markers in plant breeding may not be clearly understood by non-molecular biologists. This review provides an introduction to DNA markers and the concept of polymorphism, linkage analysis and map construction, the principles of QTL analysis and how markers may be applied in breeding programs using MAS.

RESEARCH METHODOLOGY

Plant material :

Tomato seeds were obtained from Indo American Hybrid Seeds (India) Pvt. Ltd. Bangalore.

Genomic DNA extraction :

DNA was isolated using a cetyltrimethylammonium bromide (CTAB) method (Saghai Maroof *et al*.1984).

Primer selection :

The oligonucleotide belonging to kit from Operon Technologies Inc.(Alameda, California, USA) was tested as single primers to identify the most promising detectable polymorphisms. DNA polymorphism was analyzed, on the basis of their ability to detect the DNA length polymorphisms and production of the reliable and scorable banding patterns.

PCR amplification:

A set of random operon primers was used to screen in this study. Subsequently polymerase chain reaction (PCR) was performed in 15µl reaction mixture containing 10 x *Taq* buffer containing tris with $MgCl_2$,10mM dNTPs mix, RAPD primers (100ng/ µl), DNA template (10ng/ µl), taw DNA polymerase (3U/ µl). Amplifications were performed in a mini cycler. Following thermal profile of PCR was used - 40 cycles at 94^{o}C, 45s; 35^{o}C, 60s and 72^{o}C, 90s. Before the first cycle, the temperature of 94^{o}C was maintained for 5 min and 72^{o}C maintained for 10 min at the final extension. On completion of PCR, 8µl of PCR products were resolved in 1.5 per cent agarose (SRL) gels stained with ethidium bromide using 0.5X TBE buffer in a submarine gel apparatus at 100V. The gels were visualized under ultraviolet light and photographed in a gel documentation system.

Electrophoresis for DNA markers :

It is the latest method of cultivar identification based on marker locus and protein banding. Based on the banding pattern of marker and protein the selves and off types can be differentiated and identified.

RESEARCH FINDINGS AND ANALYSIS

The salient features of the research as observed have been summarized below :

Table 1 : Primers used

Marker name	Marker type	Primer(Fw)	Primer(Rv)
TGS0001	Genome-SSR	GCGACCCTCTATTGAACTTGAAGAC	ACAAATCAAAGGAACAATTTCAA
TGS0018	Genome-SSR	GCGGCCCAAAGTATGATGTA	TCACACACACACACACACACA
TGS0019	Genome-SSR	GCGGCCCAAAGTATGATGTA	TCACACACACACACACACACA
TGS0020	Genome-SSR	GAAACACAAGAAAGGAAAACAAATGTA	TCCGTATTTATTTTTATTTTGGTCA
TGS0021	Genome-SSR	TCTTTCAACTTCTCAACTTTGGC	GCCGACTTCAAAAACTGCTC
TGS0022	Genome-SSR	GCAGTTTTGCGCACATAGAGG	TCATATATTTATGCCCGCCC
TGS0023	Genome-SSR	GTCATAGCAAAGTGTTTCAGAGAGA	TGTGTGCAAGTTTGGAAAAGA
TGS0024	Genome-SSR	GTCCAAATTAAAAACTAACCGCA	TTTCCAAAATGACCTAGCGG
TGS0025	Genome-SSR	GGGGTTTCCCGTAATGTTTT	TCTTGAAATTTTCCATCCAACA
TGS0026	Genome-SSR	CCTTGATTTTGATTCCCCAA	GAAGGTGCGTGATTTTGTGTT
TGS0027	Genome-SSR	GCCTCTTAATAGAAACTGGGGCA	TACGCCCCCTATATGCACTC
TGS0028	Genome-SSR	TGTGCGATTCCTAAAAAGGG	GTGTAGCCCATGATCCAACC
TGS0029	Genome-SSR	GGCATGCAAGCTTGTAGTTT	TCCTCTCAACTCTCAAGTCTACATCT
TGS0030	Genome-SSR	GACTCCCTGAGAAATCGTCCA	CGTAATTCCCACTTCTAACCCA
TGS0031	Genome-SSR	ATAAGTGGTTTTCCTTGGGC	GTTATCAGTCGCCAAAAGCC
TGS0032	Genome-SSR	GGTAATCAATTTTGAAGCTAAAAGC	TGGGAAGAACTCAAGTCAAAAA

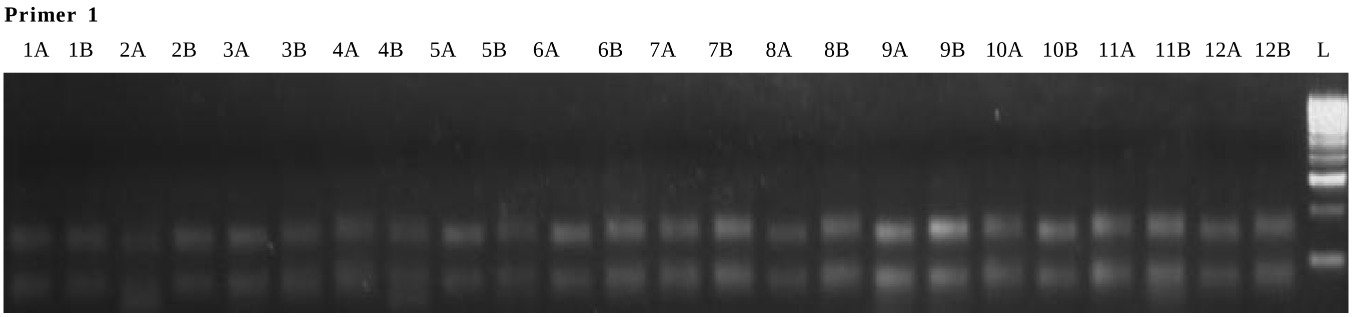

Fig. 1: Bands of genomic DNA extraction using CTAB method, run on 0.8 per cent agarose gel

Primer 2

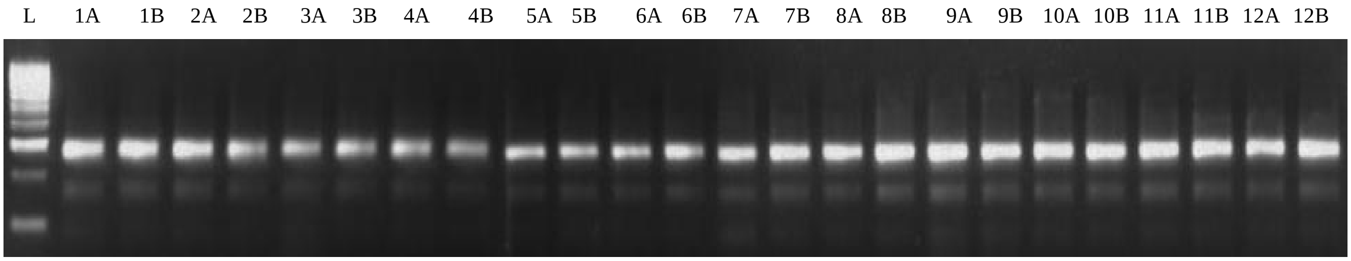

Fig. 2: Bands of genomic DNA extraction using CTAB method, run on 0.8 per cent agarose gel

Primer 3

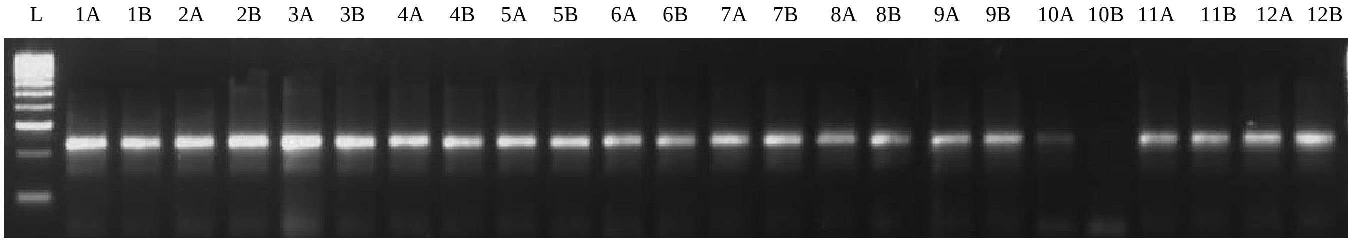

Fig. 3: Bands of genomic DNA extraction using CTAB method, run on 0.8 per cent agarose gel

Primer 4

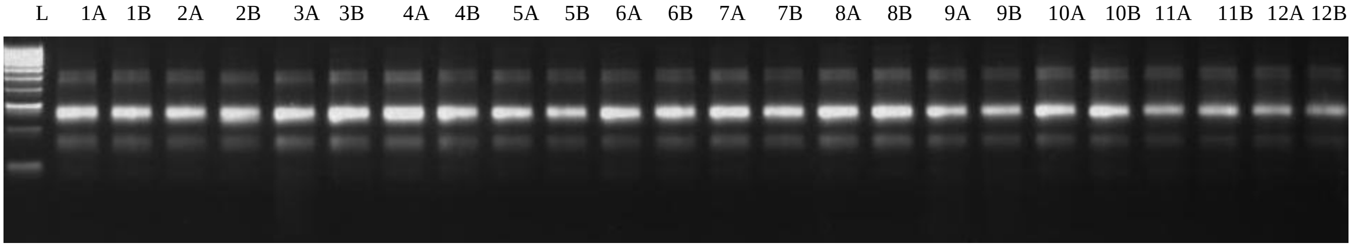

Fig. 4: Bands of genomic DNA extraction using CTAB method, run on 0.8 per cent agarose gel

Primer 5

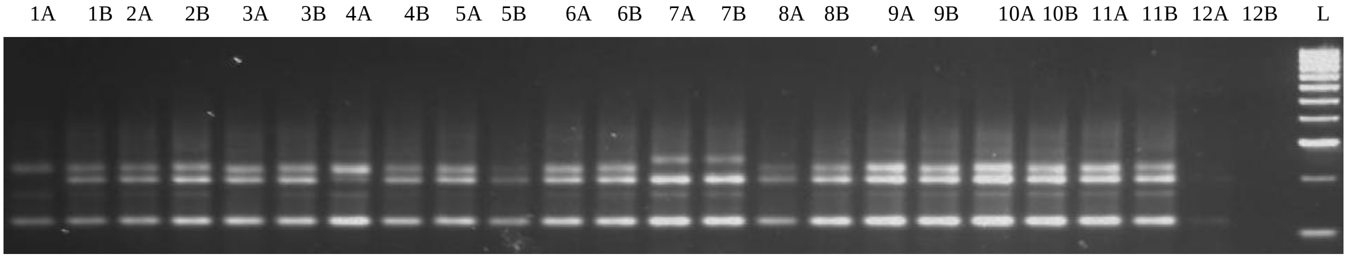

Fig. 5: Bands of genomic DNA extraction using CTAB method, run on 0.8 per cent agarose gel

Primer 6

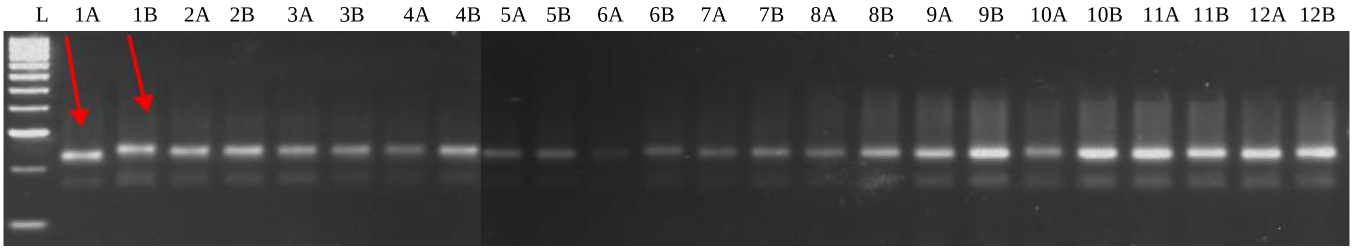

Fig. 6: SSR profile of tomato hybrid 1A and 1B shows difference in primer 6

Primer 7

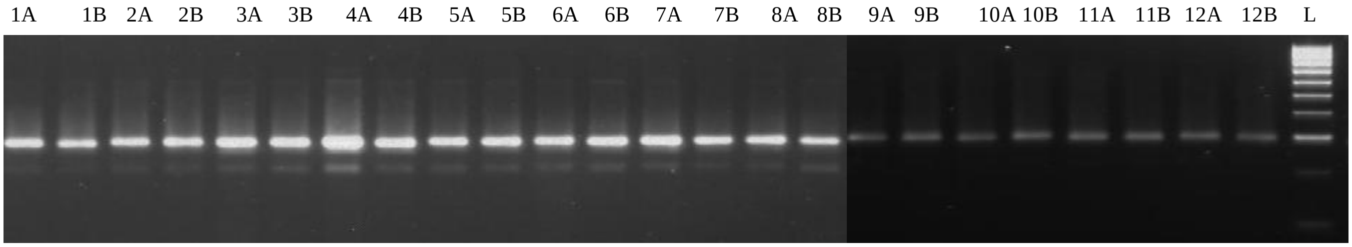
1A 1B 2A 2B 3A 3B 4A 4B 5A 5B 6A 6B 7A 7B 8A 8B 9A 9B 10A 10B 11A 11B 12A 12B L

Fig. 7: Bands of genomic DNA extraction using CTAB method, run on 0.8 per cent agarose gel

Primer 8

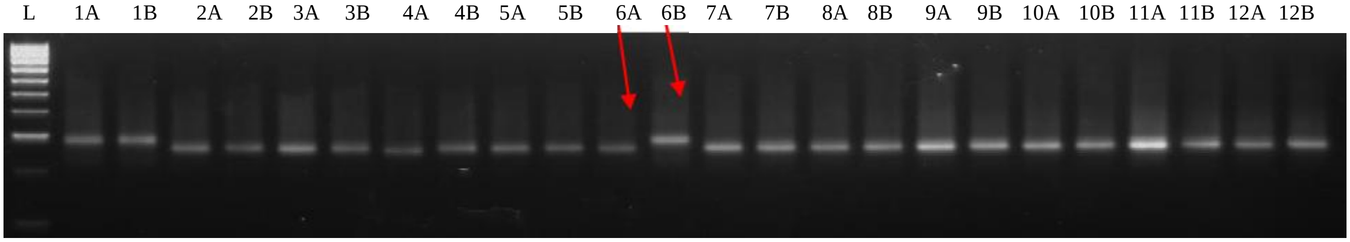
L 1A 1B 2A 2B 3A 3B 4A 4B 5A 5B 6A 6B 7A 7B 8A 8B 9A 9B 10A 10B 11A 11B 12A 12B

Fig. 8: SSR profile of tomato hybrid 6A and 6B shows difference in primer 8

Primer 9

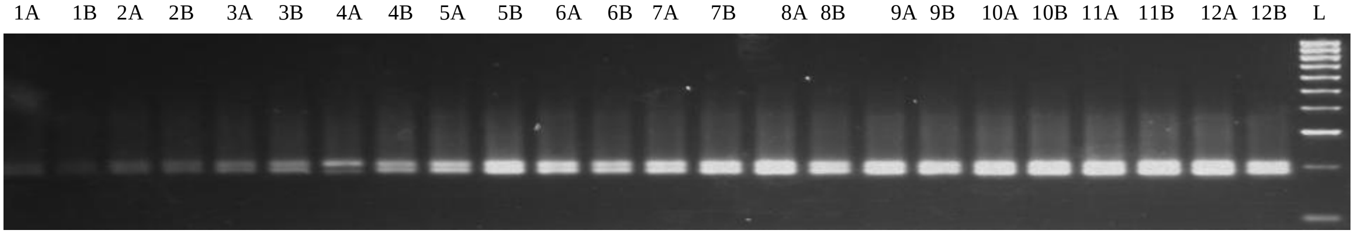
1A 1B 2A 2B 3A 3B 4A 4B 5A 5B 6A 6B 7A 7B 8A 8B 9A 9B 10A 10B 11A 11B 12A 12B L

Fig. 9: Bands of genomic DNA extraction using CTAB method, run on 0.8 per cent agarose gel

Primer 10

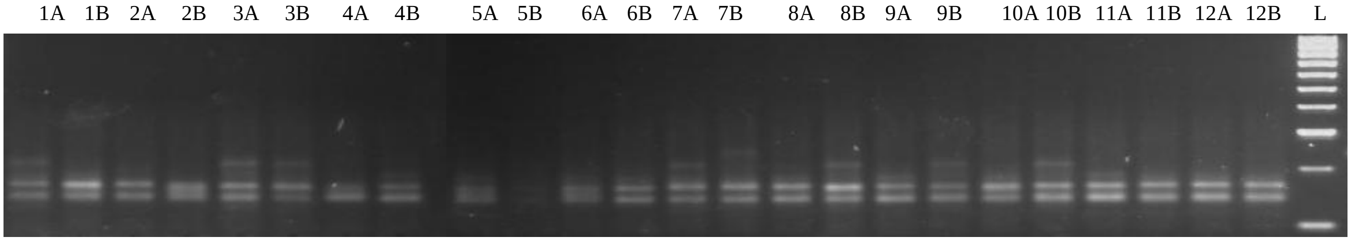
1A 1B 2A 2B 3A 3B 4A 4B 5A 5B 6A 6B 7A 7B 8A 8B 9A 9B 10A 10B 11A 11B 12A 12B L

Fig. 10: Bands of genomic DNA extraction using CTAB method, run on 0.8 per cent agarose gel

Primer 11

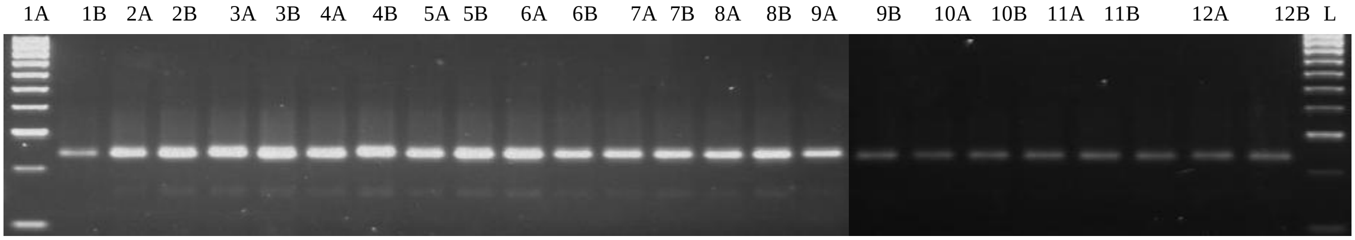
1A 1B 2A 2B 3A 3B 4A 4B 5A 5B 6A 6B 7A 7B 8A 8B 9A 9B 10A 10B 11A 11B 12A 12B L

Fig. 11: Bands of genomic DNA extraction using CTAB method, run on 0.8 per cent agarose gel

Primer 12

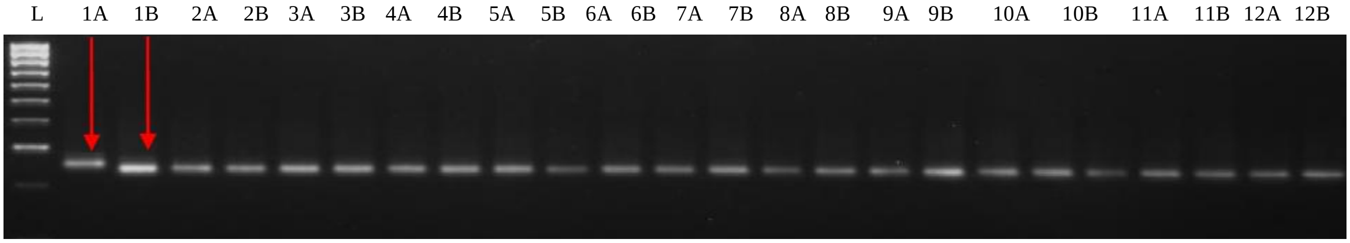
L 1A 1B 2A 2B 3A 3B 4A 4B 5A 5B 6A 6B 7A 7B 8A 8B 9A 9B 10A 10B 11A 11B 12A 12B

Fig. 12: SSR profile of tomato hybrid 1A and 2B shows difference in primer 12

Primer 13

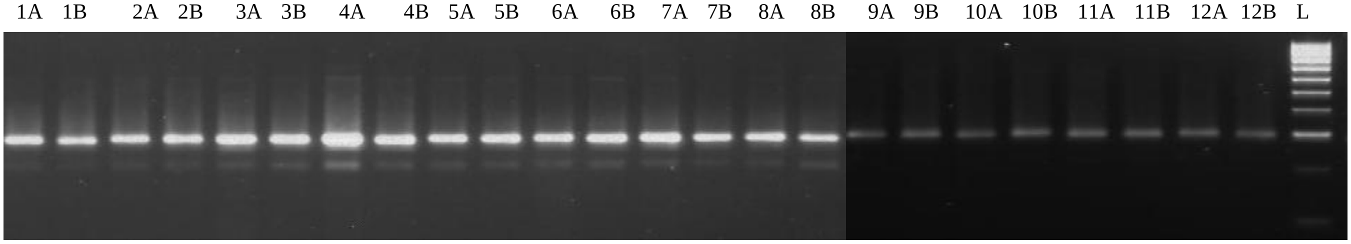
1A 1B 2A 2B 3A 3B 4A 4B 5A 5B 6A 6B 7A 7B 8A 8B 9A 9B 10A 10B 11A 11B 12A 12B L

Fig. 13: Bands of genomic DNA extraction using CTAB method, run on 0.8 per cent agarose gel

Primer 14

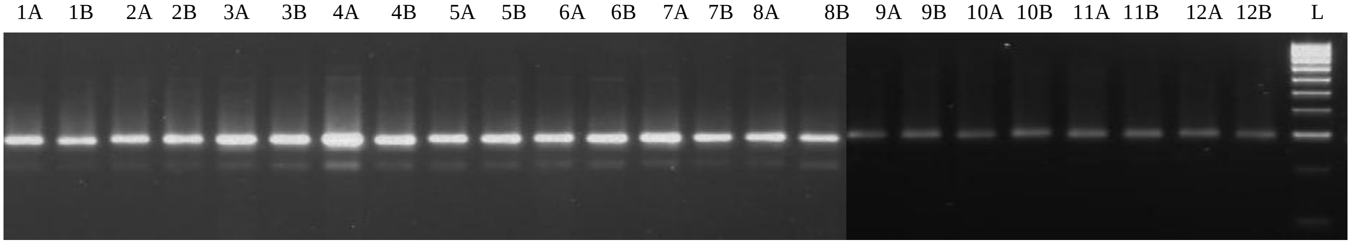
1A 1B 2A 2B 3A 3B 4A 4B 5A 5B 6A 6B 7A 7B 8A 8B 9A 9B 10A 10B 11A 11B 12A 12B L

Fig. 14: Bands of genomic DNA extraction using CTAB method, run on 0.8 per cent agarose gel

Primer 15

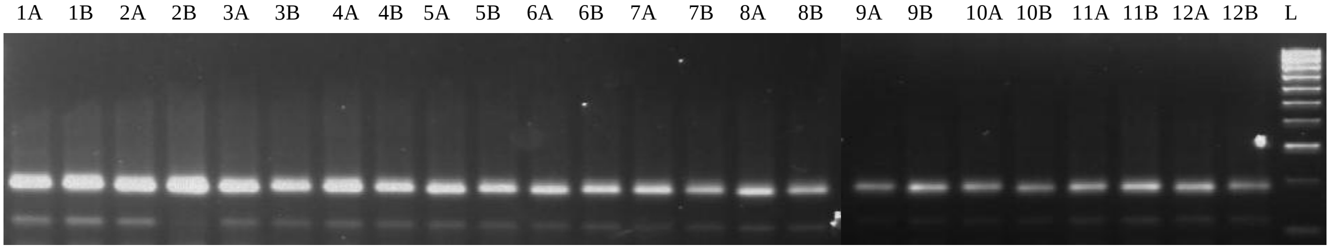
1A 1B 2A 2B 3A 3B 4A 4B 5A 5B 6A 6B 7A 7B 8A 8B 9A 9B 10A 10B 11A 11B 12A 12B L

Fig. 15: Bands of genomic DNA extraction using CTAB method, run on 0.8 per cent agarose gel

Primer 16

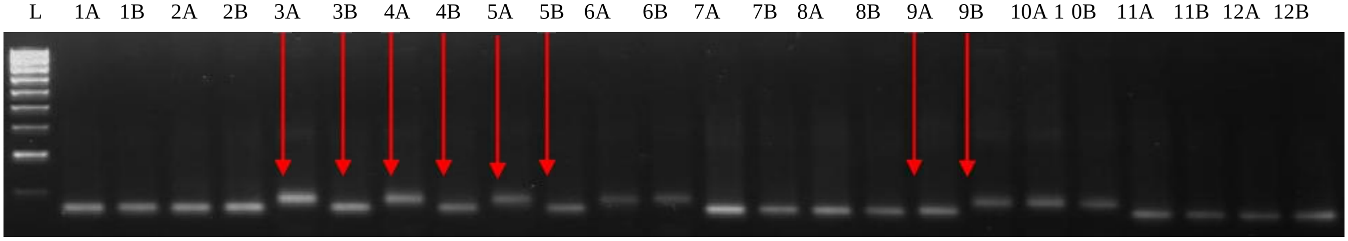
L 1A 1B 2A 2B 3A 3B 4A 4B 5A 5B 6A 6B 7A 7B 8A 8B 9A 9B 10A 1 0B 11A 11B 12A 12B

Fig. 16: SSR profile of tomato hybrid 3A,3B, 4A,4B, 5A,5B and 9A,9B shows difference in primer 16

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